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1 **In-situ biogas upgrading in thermophilic granular UASB**
2 **reactor: key factors affecting the hydrogen mass transfer rate**

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Highlights

- Biogas upgrading to 82% CH₄ is feasible in a thermophilic granular UASB reactor.
- H₂ is introduced in a separate chamber having a volume of 25% the reactor.
- H₂ low gas-liquid mass transfer rate limits the availability of H₂ for methanogens.
- H₂ distribution can be improved using porous inert devices, like ceramic sponge.
- Gas recirculation and chamber configuration help to maximize CO₂ conversion to CH₄.

Abstract

Biological biogas upgrading coupling CO₂ with external H₂ to form biomethane opens new avenues for sustainable biofuel production. For developing this technology efficient H₂ to liquid transfer is fundamental. This study proposes an innovative setup for in-situ biogas upgrading converting the CO₂ in the biogas into CH₄, via hydrogenotrophic methanogenesis. The setup consisted of a granular reactor connected to a separate chamber, where H₂ was injected. Different packing materials (rashig rings and alumina ceramic sponge) were tested to increase gas-liquid mass transfer. This aspect was optimized by liquid and gas recirculation and chamber configuration. It was shown that by distributing H₂ through a metallic diffuser followed by ceramic sponge in a separate chamber, having a volume of 25% of the reactor, and by applying a mild gas recirculation, CO₂ content in the biogas dropped from 42 to 10% and the final biogas was upgraded from 58 to 82% CH₄ content.

Keywords

In-situ biogas upgrading; Hydrogen; Gas-liquid mass transfer rate; UASB; Granules;
Anaerobic digestion

1. Introduction

Anaerobic Digestion (AD) of organic waste is a promising technology for sustainable energy production (Weiland, 2010). The potato-starch processing industry produces, as byproduct, up to 1 m³ of potato juice per ton of potatoes (Abeling and Seyfried, 1993). Potato-starch wastewater contains high concentration of biodegradable compounds, such as starch and proteins, suitable for biogas production via AD (Barampouti et al., 2005). Biogas typically contains ~50-70% CH₄ and 30-50% CO₂. Biogas upgrading to CH₄ content higher than 90% increases its heating value and its potential applications as alternative to natural gas (Deng and Hägg, 2010).

Methods currently available for biogas upgrading are mainly based on physicochemical CO₂ removal. Nevertheless, these technologies require use of additional materials and chemicals considerably increasing the cost of the process and energy input. Alternatively, biogas can be upgraded by biologically coupling H₂, derived from water electrolysis, with CO₂ present in the biogas to convert them to CH₄. H₂ can be produced using the electricity generated by the surplus of energy from wind mills or photovoltaic facilities, which may result from variable weather conditions. This reaction is carried out by a group of microorganisms known as hydrogenotrophic methanogenic archaea that utilize CO₂, as carbon source, and H₂, as electron donor, to produce CH₄ via hydrogenotrophic methanogenesis (Muñoz et al., 2015). Previous studies demonstrated that the addition of H₂ to a conventional biogas reactor can lead to

20 to 40% increase in CH₄ production rate, as result of the conversion of the CO₂ present in the biogas to additional CH₄ (Luo and Angelidaki, 2013; Luo et al., 2012).

Although biological biogas upgrading offers economical and technical advantages compared to traditional methods (Nordberg et al., 2012), H₂ mediated biogas upgrading is still challenging. One of the main limitations is the low H₂ gas-liquid mass transfer rate (Bassani et al., 2015; Luo and Angelidaki, 2012; Luo et al., 2012).

H₂ gas-liquid mass transfer rate can be described by the following equation (1):

$$r_t = 22.4k_La(H_{2gTh} - H_{2l})$$

where r_t (L/(L-day)) is the H₂ gas-liquid mass transfer rate, 22.4 (L/mol) is the gas volume to mole ratio (1 mol gas corresponds to 22.4 L at STP), k_La (day⁻¹) is the gas transfer coefficient, H_{2gTh} (mol/L) represent the H₂ concentration in the gas phase while H_{2l} (mol/L) the H₂ dissolved in the liquid phase. One way to increase H₂ gas-liquid mass transfer rate is by increasing k_La . This coefficient is specific for given reactor configuration and operating conditions (Pauss et al., 1990). Therefore, k_La can be modulated by changing parameters such as mixing speed (Bhattacharyya and Singh, 2010; Luo and Angelidaki, 2012), gas recirculation (Guiot et al., 2011) and H₂ diffusion device (Luo and Angelidaki, 2013; Díaz et al., 2015).

Besides, high-rate anaerobic treatment using up-flow anaerobic sludge blanket (UASB) reactors is commonly applied in industrial wastewater treatment plants (Gomec, 2010; Sevilla-Espinosa et al., 2010). Moreover, typically a UASB process is expected to provide higher methane content in the biogas than a CSTR process (Nizami et al., 2012).

UASB reactors' technology is based on the presence of granular sludge comprised of microorganisms responsible for catalyzing the biological conversion of organic matter

to biogas. High recirculation flow rates and consequent high up-flow velocities have an important role for the hydraulic mixing improving the wastewater to granules contact (Powar et al., 2013; Zheng et al., 2012). It has been previously reported that carbohydrate degraders and hydrogenotrophic methanogens are predominant in starch-grown granules, likely due to their role in the interspecies H_2 transfer with syntrophic bacteria (Lu et al., 2015). Moreover, previous studies on H_2 mediated biogas upgrading demonstrated that H_2 affected the microbial community composition enhancing the hydrogenotrophic methanogenic pathway and the syntrophic relationship between bacteria and hydrogenotrophic methanogens (Bassani et al., 2015).

In this study an innovative setup consisting of a UASB granular reactor connected to a separate chamber, where the H_2 was injected, was designed to mediate efficient H_2 transfer to liquid phase for biological conversion of H_2 and CO_2 to CH_4 . Key factors affecting the H_2 gas-liquid mass transfer rate were evaluated. More specifically, the effect of different operating conditions aiming in increasing $k_L a$ of H_2 to gas, and thereby increase the gas to liquid transfer, were studied to elucidate their role in improving CO_2 and H_2 conversion to CH_4 . Parameters examined were liquid and gas recirculation and configuration of diffusion devices. Moreover, the addition of packing materials as a mean to minimize the gas bubble size and thus increase the gas dissolution in the liquid was tested. Finally, the effect of gas retention time was evaluated using single or serial chamber configurations with different working volumes.

2. Materials And Methods

2.1 Substrate characteristics and feedstock preparation

Potato-starch wastewater substrate was obtained from Karup Kartoffelmelfabrik potato-starch processing factory, Denmark. Because potato-starch processing involves an up-concentration step, the provided substrate was diluted 10 times with water and Basal Anaerobic (BA) medium, to adjust the volatile solids (VS) content to the required operation conditions. Successively, the substrate was stored at -20°C, in 5 L bottles and thawed at 4°C for 2-3 days, before usage. BA medium was prepared as described in Supplementary Information (SI). The diluted substrate had a pH of 6.05, chemical oxygen demand (COD) of 21.76 ± 0.15 g/L, total solids (TS) and VS content of 26.14 ± 0.17 and 18.73 ± 0.12 g/L, respectively. The concentration of total volatile fatty acids (VFA) was 49.29 ± 4.94 mg/L. Total Kjeldahl Nitrogen (TKN) and ammonium nitrogen NH_4^{+} ($\text{NH}_4\text{-N}$) were 1.24 ± 0.01 and 0.30 ± 0.01 g-N/L, respectively.

2.2 Setup and operation of the reactors

Each setup was composed of a UASB reactor with a working volume of 1.4 L, connected to a separate H_2 -injection chamber with a working volume of 0.2 L. The feeding was introduced from the bottom of the UASB. The reactors were inoculated with 550 g of mesophilic granules, obtained from Colsen wastewater treatment plant treating potato starch wastewater (The Netherlands) and BA medium. The granules were adapted to thermophilic conditions for 25 days by feeding the reactors with diluted potato starch wastewater at hydraulic retention time (HRT) of 7 days and organic loading rate (OLR) of 2.79 gVS/L.day. A double net-separator was located in the upper part of each UASB to prevent the wash out of granules. One setup (R1) was used as upgrading reactor, while the other (R2) was utilized as control reactor operated throughout the experiment without H_2 injection. Both reactors were maintained at

thermophilic conditions (55 ± 1 °C) by circulating hot water through a water jacket around the UASB reactors glass walls.

After the startup phase, the whole experiment was divided in 8 periods. During period I the OLR was increased to 3.73 gVS/L day shortening the HRT to 5 days (Pre H₂ phase). The recirculation flow rate was set to 4 L/h. From period II, H₂ was continuously injected to R1 through a diffuser placed at the bottom of the H₂-injection chamber (In-situ phase). Rashig rings (5 mm internal diameter) were inserted into the separate chamber of both reactors to maximize the H₂ gas-liquid mass transfer rate in case of R1. The volumetric H₂ flow rate was set to 4 times the CO₂ production rate (in the gas phase) recorded before the H₂ addition, according to Luo and Angelidaki (2013b), i.e. 3.5 L/L.day, and then reduced to improve the H₂ consumption. In period III, the recirculation flow rate of both reactors was increased to 7 L/h. Successively, in period IV, rashig rings were replaced by an inert alumina ceramic sponge, while in periods V and VI different gas recirculation flow were applied. In order to evaluate the effect of the gas retention time, the H₂-injection chamber volume was doubled to 400 mL by connecting two chambers in series (Period VII) or by assembling them as a single chamber with extended length (Period VIII).

The percentage of H₂ utilized was calculated according to the following equation (2):

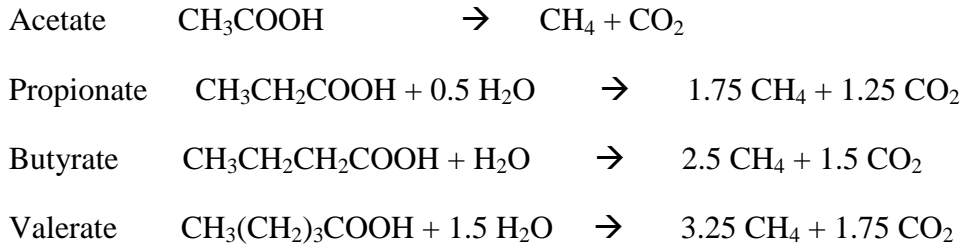
$$\text{H}_2\text{utilization efficiency} = \frac{\text{H}_2 \text{ injected } \left(\frac{\text{L}}{\text{L} - \text{day}} \right) - \text{H}_2 \text{ in biogas } \left(\frac{\text{L}}{\text{L} - \text{day}} \right)}{\text{H}_2 \text{ injected } \left(\frac{\text{L}}{\text{L} - \text{day}} \right)} * 100$$

The percentage of CH₄ derived from the conversion of CO₂ and H₂ was calculated according to the equation 3:

CH₄ from CO₂ and H₂ conversion (%) =

$$\left(\frac{(\text{CH}_4 \text{ production rate in R1 } (\frac{\text{L}}{\text{L.day}}) - \text{CH}_4 \text{ production rate in R2 } (\frac{\text{L}}{\text{L.day}}))}{\text{CH}_4 \text{ production rate in R2 } (\frac{\text{L}}{\text{L.day}}) + \text{CH}_4 \text{ production rate equivalent to VFA in R2 } (\frac{\text{L}}{\text{L.day}})} + \frac{(\text{CH}_4 \text{ production rate equivalent to VFA in R1 } (\frac{\text{L}}{\text{L.day}}) - \text{CH}_4 \text{ production rate equivalent to VFA in R2 } (\frac{\text{L}}{\text{L.day}}))}{\text{CH}_4 \text{ production rate in R2 } (\frac{\text{L}}{\text{L.day}}) + \text{CH}_4 \text{ production rate equivalent to VFA in R2 } (\frac{\text{L}}{\text{L.day}})} \right) * 100$$

Where CH₄ production rate represents the volume of CH₄ produced per liter of reactor, per day, measured at the outflow of the reactor. While CH₄ production rate equivalent to VFA was calculated converting VFA concentrations, in the reactors, to CH₄ production equivalent according the following conversion reactions:



This was done to take into account the biomethanation inhibition caused by the injection of H₂ in the upgrading reactor and provide a more accurate estimation of the CH₄ produced from the conversion of CO₂ and H₂.

2.3 Analytical methods

The biogas production was recorded in daily basis. TS, VS, NH₄-N and TKN were measured according to the Standard Methods for Examination of Water and Wastewater (APHA, 2005). Liquid samples from the reactors were collected for pH and VFA analysis every second day. VFA and pH were measured according to Kougias et al., (2015) as described in SI. Detailed description of chromatographs utilized to measure biogas composition and CH₄ production (for batch assays) are given in SI. Detection limits for the measurement of CH₄, CO₂ and H₂ by GC were defined by the calibration curve (5–100%), while the detection limits for VFA were 5–1500 mg/L.

2.4 Specific methanogenic activity test

Specific methanogenic activity (SMA) assays were conducted during reactors' steady state operation. 1 g of granules and 9 mL of liquid sample obtained from the reactors were immediately transferred to 36 ml serum bottles under anaerobic conditions. The bottles were supplemented with acetate (20 mM) or H₂/CO₂ (80:20, 1 atm). Bottles with glucose (10 mM) or water as substrate were prepared as control and blank, respectively. All the tests were prepared in triplicates, flushed with N₂, sealed with rubber stoppers and aluminum caps and incubated at 55 °C and 155 rpm.

3. Results And Discussion

3.1 Process performances and biogas upgrade

Operational data from upgrading (R1) and control (R2) reactor under steady state conditions are reported in Table 1 and 2.

3.1.1 Period I: the pre H₂ phase

In the pre H₂ phase (Period I), the two reactors showed similar performance in terms of biogas production rate (on average 2147 mL/L-reactor.day) and CH₄ yield (335 mL/gVS, corresponding to ~70% of the theoretical) (Table 1). This result is in accordance with previous studies on biogas production from starch biomasses (Frigon and Guiot, 2010). The average CH₄ content of the reactors was ~59% (Table 1 and Fig. 1), the pH was ~7.5 and the total VFA content >1 g/L (Table 1 and Fig. 2).

3.1.2 Period II: effect of rashig rings as H₂ distribution device on biogas upgrading performance

To increase the $k_L a$ and thereby enhance gas-liquid transfer, rashig rings were placed in the H₂-injection chamber to break H₂ bubbles and thus increase contact surface area between gas and liquid phases (Kramer and Bailey, 1991). Once steady state conditions were achieved, H₂ was continuously injected (3.5 L/L.day), through a metallic diffuser, in the H₂-injection chamber (In-situ phase). By comparing reactors' performance, in R1, 45% higher CH₄ production rate was observed (Table 1 and Fig. 3). Additionally, a pH increase to 7.9 was recorded in R1, as a result of the CO₂ removal (Table 1 and Fig. 2a). Nevertheless, because of the low H₂ gas-liquid mass transfer rate, only 51% of the H₂ injected was utilized leading to a high amount of unutilized H₂ in the output gas (45%) (Table 1 and Fig 1a). Additionally, a remarkable increase in VFA levels, reaching 3.4 g/L, was recorded in the upgrading reactor, while VFA concentration in the control reactor remained stable (Table 1 and Fig. 2b). This is likely due to the high H₂ partial pressure that affected negatively acidogenic VFA conversion resulting in their accumulation. Moreover, the continuous H₂ injection led to a progressive higher H₂ partial pressure, which shifted the metabolic pathway towards homoacetogenesis inhibiting methanogenesis (Cord-Ruwisch et al., 1997). This argument was supported by the predominance and accumulation of acetate over other VFA in R1 accounting for 55% of total VFA (Table 1). Moreover, this level was 4 % higher than the correspondent level in R2, which, together with higher total VFA concentrations, demonstrates the instability caused by the excessive H₂ flow rate provided in R1. Therefore, to provide a more accurate estimation of the increment of the CH₄ production rate due to CO₂ and H₂ conversion, the total VFA concentrations in the two systems

were converted in equivalent CH_4 production, as described in section 2.2. The difference in the VFA concentration between the two reactors was taken into account to estimate the inhibition of liquid substrate degradation occurring in the upgrading reactor and allow the reactors' performances to be comparable. Thus, the CH_4 derived from CO_2 and H_2 conversion was calculated (equation 3) based on the difference between the CH_4 production rates of the two systems after normalization of VFA.

To overcome the negative effect of the H_2 on the biomethanation process and improve the H_2 consumption, in the last part of this period the H_2 flow rate was reduced to 2.6 L/L.day reducing the unutilized H_2 to 34% of the output gas and increasing the CH_4 content to 47%.

3.1.3 Period III: effect of liquid recirculation on upgrading performance

Good mixing is known to be crucial to make substrates available for microorganisms (Bhattacharyya and Singh, 2010; Luo and Angelidaki, 2012). Moreover good mixing increases the $k_L a$ for gasses, which is function of the surface area per unit volume, thereby increasing gas-liquid contact (Kramer and Bailey, 1991). Therefore, to improve H_2 -liquid contact, the liquid recirculation flow was increased from 4 to 7 L/h, while the H_2 flow rate was maintained to 2.6 L/L.day leading to a slight increase of the utilized H_2 (53%) (Table1). The unutilized H_2 and the CH_4 content in the output gas stabilized to 37% and 45%, respectively (Table 1 and Fig. 1a). Similarly, in this period in R1 36% higher CH_4 production rate was recorded, compared to R2 (Table 1 and Fig. 3). As these results did not markedly differ from the last part of period I (i.e. H_2 flow rate was reduced to 2.6 L/L.day), it can be concluded that the improved upgrading efficiency was mainly attributed to the lower H_2 flow rate applied, rather than to the higher liquid

recirculation flow. In fact, upon H₂ addition, the granular bed appeared less expanded, probably due to reduced dissolved CO₂ concentration in the liquid, due to the hydrogenotrophic consumption of CO₂ to CH₄ (Ohsumi et al., 1992; Song et al., 2005). Therefore, the positive effect of the higher liquid recirculation on biogas production and upgrading was not achieved.

3.1.4 Period IV: effect of alumina ceramic sponge as H₂ distribution device on upgrading performance

An alternative method to reduce H₂ bubbles size and thus increase gas-liquid contact is by increasing the surface area of the material over which the bubbles travelled and thereby breaking them to a smaller size. Based on that, the rashig rings in the H₂-injection chamber were replaced with alumina ceramic sponge. Alumina ceramic sponge introduced in the chamber had 16 m² (0.3 m²/g) surface area which is significantly higher compared to the surface area in rashig rings (0.1 m², corresponding to 0.002 m²/g). Interestingly, in this period, the H₂ utilization and the CH₄ production rate derived from CO₂ and H₂ conversion increased (Table 1 and Fig. 3). On average, 67% of the H₂ injected was utilized reducing the H₂ content in the output gas to 31% and increasing the CH₄ content to 52% (Table 1 and Fig. 1a). These results clearly show the influence of the H₂ distribution on the upgrading performances indicating the importance of porosity and pore size of the H₂ distribution device for an effective H₂ utilization by microorganisms.

In this period lower biogas and CH₄ production rates were observed in particular in R2 (Table 1 and Fig. 3). Previous studies have demonstrated that aluminum oxide does not cause any toxic effects on microorganisms' growth (Ingham et al., 2012).

Additionally, state indicators of the biomethanation process, such as VFA and pH, did not demonstrate any imbalance. More specifically, the VFA levels recorded in this period and particularly for R1 were at the lowest levels compared to the other periods (Table 1 and Fig. 2b). Therefore, we assume that ceramic sponge pores could have retained undigested biomass particles with consequent decrease of CH₄ production.

In the last part of this period, in order to reduce the unutilized H₂, the H₂ flow rate was further decreased to 2 L/L.day resulting in reduced H₂ and increased CH₄ content in the output gas to 20% and 57%, respectively.

3.1.5 Period V and VI: effect of gas recirculation on upgrading performance

As previously described, gas recirculation would have a positive effect on $k_L a$ coefficient, increasing H₂ gas-liquid mass transfer rate (Equation 1) (Guiot et al., 2011).

Therefore, in period V, 4 mL/min gas recirculation (then increased to 6 mL/min, in period VI) were applied to R1 improving the H₂ dissolution and thus significantly increasing the CO₂ conversion. In fact, in these periods on average 87% of the H₂ injected was utilized leading to 37% higher CH₄ production rate (Table 2 and Fig. 3). Nevertheless, an increase in the pH value to 8.2 was recorded as a result of the CO₂ removal (Table 2 and Fig. 2a). The CH₄ content in the biogas markedly increased to 66% and the unutilized H₂ decreased to 14% (Table 2 and Fig. 1a). To further decrease the unutilized H₂, at the end of the period the H₂ flow rate was reduced to 1.8 L/L.day (corresponding to ~2.5 times the CO₂ production rate recorded in R2). Nevertheless, no substantial difference in biogas composition and upgrading performances was recorded. In previous studies, H₂ distribution in the reactor's liquid phase was optimized by the application of gas recirculation flow rates ~4-folds higher than the input gas flow rate

(Díaz et al., 2015). Unfortunately, in this experiment, beside the positive effect on upgrading performances, the application of such a high gas recirculation flow rate led to an excessive pressure through the diffuser and to turbulent movements causing granules disintegration. The subsequent reduction of reactor's active biomass can explain the lower CH₄ production rate and VFA levels higher than 5 g/L observed in R1 from period V (Table 2, Fig. 2b and Fig. 3).

3.1.6 Period VII and VIII: Effect of gas retention time using H₂-injection chamber configuration on upgrading process performance

To increase the contact area between H₂ bubbles and liquid, and therefore increase H₂ transfer coefficient (Equation 1), the ceramic sponge surface area was doubled. This was done by doubling H₂-injection chamber volume, either by connecting two chambers in series (Period VII), or by assembling them in a single longer chamber (Period VIII). The connection of two chambers in series did not lead to a substantial improvement of upgrading performances, indicating that chamber's volume itself has not a direct correlation with H₂ distribution. Nevertheless, by assembling two chambers in a single longer one, a higher H₂ percentage was utilized (94%) resulting in only 8% H₂ unutilized (Table 2 and Fig. 1a). Therefore, CO₂ and CH₄ contents in the output biogas dropped to 10% and increased to 81% (with a maximum of 82%) respectively (Table 2 and Fig. 1a). However, in this period the pH raised to 8.4 as a consequence of the high CO₂ conversion (Table 2 and Fig. 2a). The results clearly demonstrate the importance of a proper reactor configuration design that increases the gas retention time leading to more efficient H₂ distribution and CO₂ conversion to CH₄.

Moreover, from the comparison of reactors CH_4 production rate, it was shown that, in the upgrading reactor, on average the CH_4 produced from the conversion of CO_2 represented ~37% of the total recorded CH_4 production rate (Table 1 and 2 and Fig. 3).

Finally, it should be mentioned that the lower CH_4 production and higher VFA levels of control reactor observed in period VII were due to the disassembly of the separate chamber in order to be mounted in the upgrading reactor (Table 2 and Fig. 2b and 3). The CH_4 productivity and the VFA concentration of the control reactor were recovered in period VIII.

3.2 Specific methanogenic activity test

H_2 addition is known to promote the hydrogenotrophic methanogenic pathway (Bassani et al., 2015; Luo and Angelidaki, 2013a, 2013b). Therefore, in this experiment, SMA tests were performed to validate the effect of the H_2 addition on methanogenesis pathways. Granules and liquid samples were taken from the reactors at steady state of periods IV (introduction of ceramic sponge as H_2 distribution device) and V (application of gas recirculation). It was shown that the preferable methanogenic pathway in both reactors (i.e. R1 and R2) was hydrogenotrophic (Table 3). This result was expected because hydrogenotrophic methanogens are known to be predominant in starch-grown granules (Lu et al., 2015).

In period IV, CH_4 production rate achieved by batches fed with H_2/CO_2 did not show markedly difference between the two reactors. Conversely, in period V, higher hydrogenotrophic activity was observed in R1 compared to the control reactor, likely due to the gas recirculation enhancing the effect of H_2 addition on microbial community composition and thus stimulating hydrogenotrophic methanogenic pathway.

Both tests showed low acetoclastic activity which can be explained by the high acetate levels detected in the reactors before the tests which further increased in period V (~3.3 g/L in R1 and ~1.5 g/L in R2; Table 2). Moreover, by comparing the concentration of unutilized acetate at the end of SMA tests and in the UASB reactors, it was shown that acetate levels markedly decreased in all batches (from 3 to 2.5 g/L in the upgrading system and from 1.4 to 1.3 g/L in the control treatment), apart from batches fed with acetate, where acetate levels increased to 3.3 and 1.8 g/L in R1 and R2, respectively. These results indicate that high acetate levels in the inoculum obtained from the reactor probably inhibited the process not allowing the further degradation of the supplemental amount of acetate that was added in the batch bottles (Gorris et al., 1989). Finally, it was found that the specific microbial activity for the degradation of glucose was lower in period V compared to period IV. This could be possibly due to the negative effect of gas recirculation on the granules as previously discussed in the continuous reactor operation (Tables 1, 2 and 3).

4. Conclusions

The current research demonstrated the feasibility of in-situ biogas upgrading using an external chamber with 25% of the conventional biogas reactor volume. Key factors affecting the H₂ gas-liquid mass transfer rate were tested to improve the efficiency of the overall process. It was shown that the use of porous devices benefit the H₂ uptake as the active contact area is increasing and the gas retention time is extended. Moreover, the gas recirculation flow rate and the chamber design are fundamental elements that must be considered to maximize the gas retention time and thus the H₂ dissolution to the liquid media.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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458 **Table captions:**

459 **Table 1:** Upgrading (R1) and control (R2) reactor performances under steady state
460 conditions (Periods I-IV).

461 **Table 2:** Upgrading (R1) and control (R2) reactor performances under steady state
462 conditions (Periods V-VIII).

463 **Table 3:** Specific methanogenic activity (SMA) results, expressed as CH₄ production
464 rate (mL/L.day), under steady state conditions.

465 **Figure captions:**

466 **Fig. 1:** Biogas composition (CH_4 (●), CO_2 (○) and H_2 (■) %) of (a) upgrading and (b)
467 control reactor.

468 **Fig. 2:** pH (a) and total VFA (b) of upgrading (◆) and control (○) reactor.

469 **Fig. 3:** CH_4 production rate of upgrading (◆) and control (○) reactor.

Table 1

Phase	Pre H ₂				In-situ			
Period	I		II		III		IV	
H ₂ distribution device	-		rashig rings		rashig rings		ceramic sponge	
Reactor	R1	R2	R1	R2	R1	R2	R1	R2
Liquid recirculation flow (L/h)	4	4	4	4	7	7	7	7
Gas recirculation flow (mL/min)	NA*	/	NA*	/	NA*	/	NA*	/
Biogas production rate (mL/L.day)	2167±180	2127±180	2093±232	2229±129	2072±102	2015±75	1953±97	1787±57
Biogas composition (%):								
CH ₄	58.2±3.4	60.3±3.0	40.4±4.3	60.6±1.8	44.9±2.3	60.9±1.0	52.0±1.9	62.5±0.3
CO ₂	41.8±3.4	39.7±3.0	14.9±3.2	39.4±1.8	18.5±3.2	39.1±1.0	17.0±0.7	37.5±0.3
H ₂	NA*	/	44.6±6.7	/	36.6±1.9	/	31.0±1.9	/
CH ₄ production rate (mL/L.day)	1255±54	1277±61	1528±147	1350±74	1497±73	1227±53	1471±72	1117±39
CO ₂ in output gas (mL/L.day)	912±148	850±134	565±115	878±73	618±55	789±33	482±34	670±19

H ₂ flow rate (mL/L.day)	NA*	/	3477±594	/	2636±89	/	2629±93	/
H ₂ consumption rate (mL/L.day)	NA*	/	1769±330	/	1412±212	/	1756±121	/
pH	7.46±0.03	7.49±0.06	7.92±0.11	7.59±0.09	7.90±0.06	7.60±0.05	7.93±0.12	7.56±0.09
Total VFA (g/L)	1.69±0.37	1.21±0.25	3.40±0.31	1.41±0.28	3.60±0.23	2.26±0.11	2.81±0.46	2.37±0.32
Acetate content in VFA (%)	41.3±4.3	49.0±3.9	55.3±4.0	51.5±3.8	51.8±2.3	47.3±3.7	49.7±3.8	47.2±4.2

*NA: not applicable to this period

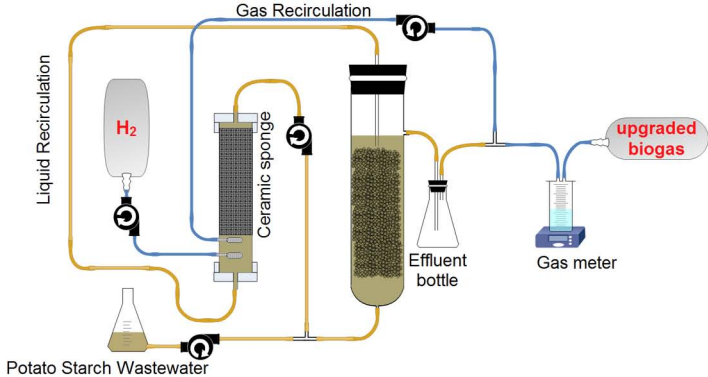
Table 2

Phase	In-situ							
Period	V		VI		VII		VIII	
H ₂ distribution device	ceramic sponge		ceramic sponge		serial chambers		single chamber with extended length	
Reactor	R1	R2	R1	R2	R1	R2	R1	R2
Liquid recirculation flow (L/h)	7	7	7	7	7	7	7	7
Gas recirculation flow (mL/min)	4	/	6	/	6	/	6	/
Biogas production rate (mL/L.day)	1786±68	1900±85	1521±98	2018±275	1337±72	1175±138	1261±157	1558±188
Biogas composition (%):								
CH ₄	66.4±1.9	61.1±1.2	66.0±2.5	65.0±2.4	67.6±2.0	65.0±1.0	81.3±0.6	66.7±2.8
CO ₂	20.5±4.0	38.9±1.2	18.35±3.9	35.0±2.4	18.8±0.5	35.0±1.0	10.2±1.0	33.2±2.8
H ₂	13.0±4.3	/	15.7±1.4	/	13.5±2.4	/	8.5±1.5	/
CH ₄ production rate (mL/L.day)	1365±52	1161±55	1188±55	1308±149	1046±57	763±92	1145±134	1039±121

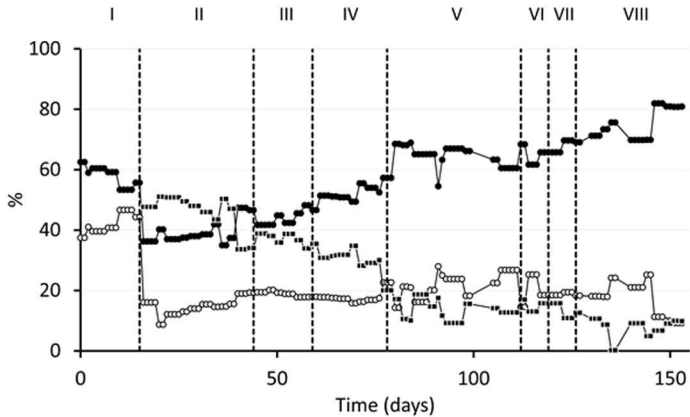
CO ₂ in output gas (mL/L.day)	421±65	740±47	333±82	710±134	291±16	412±48	121±21	615±83
H ₂ flow rate (mL/L.day)	2144±312	/	1834±30	/	1768±55	/	1828±14	/
H ₂ consumption rate (mL/L.day)	1873±234	/	1551±44	/	1536±80	/	1717±23	/
pH	7.83±0.10	7.64±0.07	8.24±0.20	7.85±0.12	8.18±0.08	7.92±0.07	8.38±0.07	7.99±0.09
Total VFA (g/L)	5.11±0.06	3.24±0.48	3.66±0.97	2.37±0.27	4.34±0.40	3.21±0.39	3.87±0.40	2.36±0.15
Acetate content in VFA (%)	64.6±3.4	46.0±4.7	39.9±2.6	39.4±4.3	37.0±2.2	36.5±2.9	30.3±1.4	34.5±6.5

Table 3

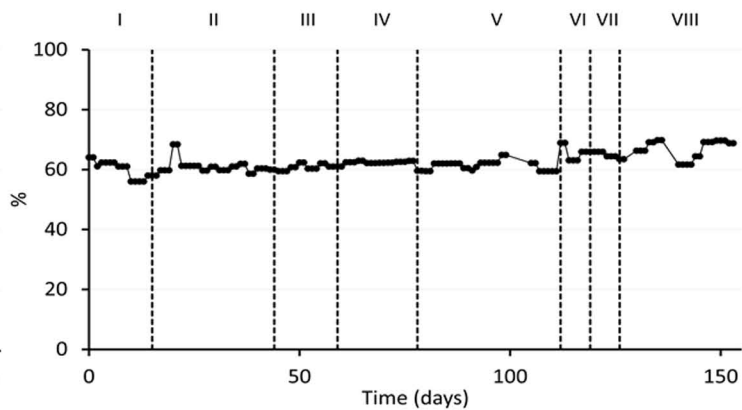
Period	IV		V	
Reactor	R1	R2	R1	R2
Blank	36±2	11±2	6±1	7±1
Glucose	589±67	219±6	73±22	23±12
Acetate	159±4	4±1	4±1	3±2
H ₂ /CO ₂	1270±20	1296±29	986±212	520±65



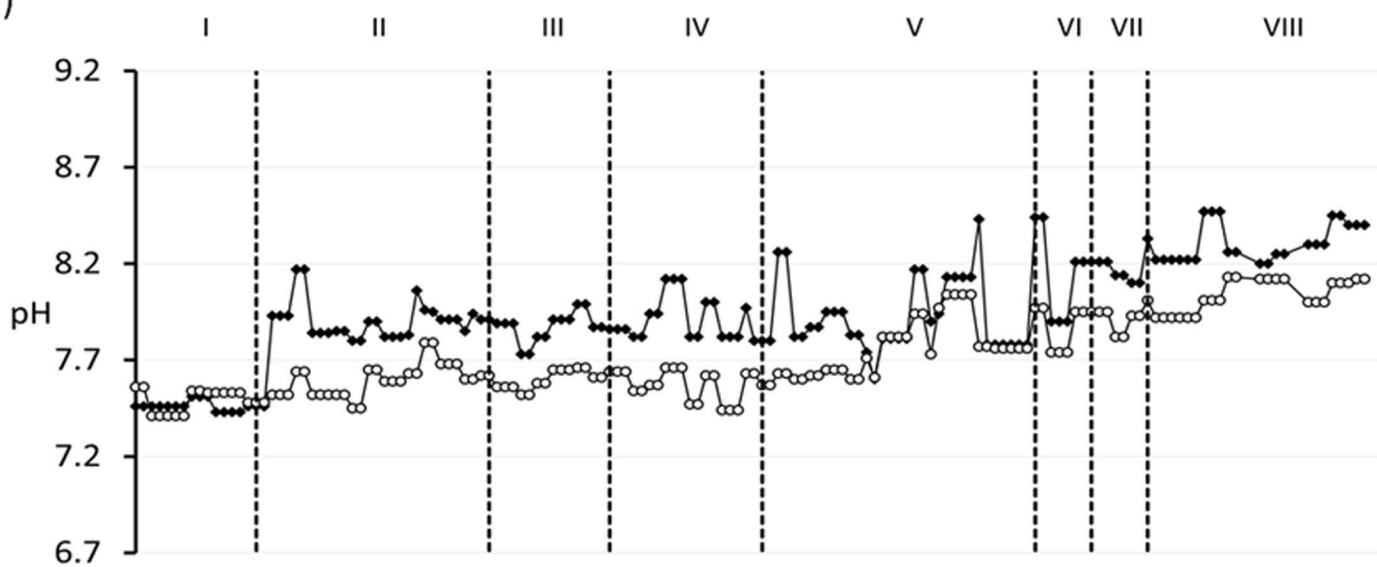
a)



b)



a)



b)

